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Editorial Review

Anti-inflammatory actions of glucocorticoids: molecular mechanisms

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1. Glucocorticoids are widely used for the suppression of inflammation in chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases, all of which are associated with increased expression of inflammatory genes. The molecular mechanisms involved in this anti-inflammatory action of glucocorticoids is discussed, particularly in asthma, which accounts for the highest clinical use of these agents.

2. Glucocorticoids bind to glucocorticoid receptors in the cytoplasm which then dimerize and translocate to the nucleus, where they bind to glucocorticoid response elements (GRE) on glucocorticoid-responsive genes, resulting in increased transcription. Glucocorticoids may increase the transcription of genes coding for anti-inflammatory proteins, including lipocortin-1, interleukin-10, interleukin-1 receptor antagonist and neutral endopeptidase, but this is unlikely to account for all of the widespread anti-inflammatory actions of glucocorticoids.

3. The most striking effect of glucocorticoids is to inhibit the expression of multiple inflammatory genes (cytokines, enzymes, receptors and adhesion molecules). This cannot be due to a direct interaction between glucocorticoid receptors and GRE, as these binding sites are absent from the promoter regions of most inflammatory genes. It is more likely to be due to a direct inhibitory interaction between activated glucocorticoid receptors and activated transcription factors, such as nuclear factor-κB and activator protein-1, which regulate the inflammatory gene expression.

4. It is increasingly recognized that glucocorticoids change the chromatin structure. Glucocorticoid receptors also interact with CREB-binding protein (CBP), which acts as a co-activator of transcription, binding several other transcription factors that compete for binding sites on this molecule. Increased transcription is associated with uncoiling of DNA wound around histone and this is secondary to acetylation of the histone residues by the enzymic action of CBP. Glucocorticoids may lead to deacetylation of histone, resulting in tighter coiling of DNA and reduced access of transcription factors to their binding sites, thereby suppressing gene expression.

5. Rarely patients with chronic inflammatory diseases fail to respond to glucocorticoids, although endocrine function of steroids is preserved. This may be due to excessive formation of activator protein-1 at the inflammatory site, which consumes activated glucocorticoid receptors so that they are not available for suppressing inflammatory genes.

6. This new understanding of glucocorticoid mechanisms may lead to the development of novel steroids with less risk of side effects (which are due to the endocrine and metabolic actions of steroids). 'Dissociated' steroids which are more active in transrepression (interaction with transcription factors) than transactivation (GRE binding) have now been developed. Some of the transcription factors that are inhibited by glucocorticoid, such as nuclear factor-κB, are also targets for novel anti-inflammatory therapies.

INTRODUCTION

Glucocorticosteroids suppress inflammation in a wide variety of diseases, including allergic diseases, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases. Indeed they are often the most effective therapy available and their use is limited only by systemic side effects. The most widespread use of glucocorticoids is in asthma and inhaled glucocorticoids have revolutionized treatment and now become

Key words: asthma, glucocorticoid, inflammation, transcription factor.

Abbreviations: AP-1, activator protein-1; CBP, CREB binding protein; COX-2, inducible cyclo-oxygenase; CREB, cyclic AMP responsive element binding protein; FGR, familial glucocorticoid resistance; GCR, glucocorticoid resistant; GCS, glucocorticoid sensitive; GM-CSF, granulocyte–macrophage colony-stimulating factor; GR, glucocorticoid receptor; GRE, glucocorticoid response elements; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; iNOS, inducible nitric oxide synthase; NF-AT, nuclear factor of activated T-cells; NF-κB, nuclear factor-κB; NO, nitric oxide; PLA_2, phospholipase A_2; STAT, signal transducer and activator of transcription; TNF-α, tumour necrosis factor-α.

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the mainstay of therapy for patients with chronic disease [1]. There have been important advances in our understanding of how glucocorticoids suppress inflammation and this may point the way to the development of improved glucocorticoids and more specific therapies in the future [2,3]. In this review I have focused on asthma as an example of an inflammatory disease that is suppressed by glucocorticoids. Asthma is the commonest inflammatory disease world-wide and accounts for by far the greatest amount of prescribed glucocorticoids.

GLUCOCORTICOID RECEPTORS

Glucocorticoids exert their effects by binding to a glucocorticoid receptor (GR) localized in the cytoplasm of target cells. There is a single class of GR that binds glucocorticoids, with no evidence for subtypes of differing affinity in different tissues. Recently a splice variant of GR, termed GR-β, has been identified that does not bind glucocorticoids but binds to DNA and may therefore potentially interfere with the action of glucocorticoids [4]. The structure of GR has been elucidated using site-directed mutagenesis, which has revealed distinct domains [5]. The glucocorticoid binding domain is at the C-terminal end of the molecule and in the middle of the molecule are two finger-like projections that interact with DNA. Each of these 'zinc fingers' is formed by a zinc molecule bound to four cysteine residues (Figure 1). An N-terminal domain (τ_1) is involved in transcriptional *trans*-activation of genes once binding to DNA has occurred and this region may also be involved in binding to other transcription factors. This is the least conserved part of the molecule between individuals and between species. Deletion analysis has demonstrated a 41-amino-acid core at the C-terminal end of the τ_1 domain that is critical for *trans*-activation. In human GR there is another *trans*-activating domain (τ_2) adjacent to the glucocorticoid binding domain and this region is also important for the nuclear translocation of the receptor. GR is phosphorylated (predominantly on serine residues at the N terminal), but the role of phosphorylation in glucocorticoid actions is not yet certain.

The inactivated GR is bound to a protein complex (approx. 300 kDa) which includes two molecules of 90 kDa heat shock protein (hsp90), a 59 kDa immunophilin protein and various other inhibitory proteins. The hsp90 molecules act as a 'molecular chaperone', preventing the unoccupied GR localizing to the nuclear compartment. Once the glucocorticoid binds to GR, hsp90 dissociates, thus exposing two nuclear localization signals and allowing the activated GR–glucocorticoid complex to rapidly move into the nucleus and bind to DNA (Figure 2).

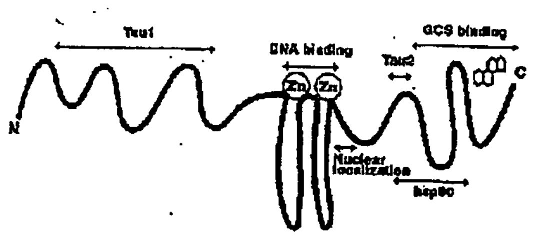

Figure 1 Domains of the GR

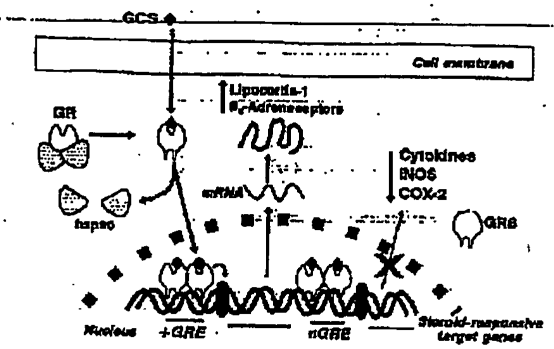

Figure 2 Classical model of glucocorticoid action

The glucocorticoid enters the cell and binds to a cytoplasmic glucocorticoid receptor (GR) that is complexed with two molecules of a 90 kDa heat shock protein (hsp90). GR translocates to the nucleus where, as a dimer, it binds to a glucocorticoid recognition sequence (GRE) on the 5′-upstream promoter sequence of glucocorticoid-responsive genes. GREs may increase transcription and negative (n)GREs may decrease transcription, resulting in increased or decreased mRNA and protein synthesis.

EFFECTS ON GENE TRANSCRIPTION

Glucocorticoids produce their effect on responsive cells by activating GR to directly or indirectly regulate the transcription of certain target genes [6,7]. The number of genes per cell directly regulated by glucocorticoids is estimated to be between 10 and 100, but many genes are indirectly regulated through an interaction with other transcription factors, as discussed below. Upon activation GR forms a homodimer which binds to DNA at consensus sites termed glucocorticoid response elements (GRE) in the 5′-upstream promoter region of glucocorticoid-responsive genes. The way in which activated GR seeks out the small number of GREs in approximately 100 000 genes is not completely understood, but recent evidence suggests that the GR dimer binds non-specifically to DNA then attaches to another strand of DNA before dissociating from the first site of attachment. This is repeated until a high-affinity GRE site is encountered, the GR thus moving through the genome like Tarzan swinging through the jungle [8]. The binding of the GR dimer to GRE changes the rate of transcription, resulting in either induction or repression of the gene. The consensus sequence for GRE binding is the palindromic 15-bp sequence GGTACAnnnTGTTCT (where n is any nucleotide), although for repression of transcription the putative negative GRE has a more variable sequence. Negative GREs have only rarely been identified in some genes, such as the pro-opiomelanocortin gene [9]. The repression of the osteocalcin gene

by glucocorticoids appears to be due to binding of the GR dimer to a GRE which overlaps the TATA box and therefore interferes with the initiation of transcription [10]. Most genes that are repressed by glucocorticoids have no GRE, however, suggesting that some other mechanism must be invoked.

Crystallographic studies indicate that the zinc finger binding to DNA occurs within the major groove of DNA with one finger from each component of the dimer interacting with one half of the palindrome [11]. In contrast to these simple GREs, there are 'composite' GREs that do not share these GRE sequences, but depend on the presence of other transcription factors binding to DNA [12]. GR may also bind to less well-defined regions of DNA and regulate promoters that contain no obvious GRE sequences. Interaction with other transcription factors may also be important in determining differential glucocorticoid responsiveness in different cell types. Other transcription factors binding in the vicinity of GRE may have a powerful influence on glucocorticoid inducibility and the relative abundance of different transcription factors may contribute to the glucocorticoid responsiveness of a particular cell type.

GR may also inhibit protein synthesis by reducing the stability of mRNA via enhanced transcription of specific ribonucleases that break down mRNA containing constitutive AU-rich sequences in the untranslated 3′-region, thus shortening the turnover time of mRNA. This is a mechanism whereby glucocorticoids inhibit the synthesis of the cytokine granulocyte–macrophage colony-stimulating factor (GM-CSF), which plays a key role in the survival of inflammatory cells at the site of inflammation [13]. Such a mechanism may also be important for the repressive effect of glucocorticoids on inducible cyclo-oxygenase (COX-2) [14]. This may be an important mechanism whereby glucocorticoids inhibit some inflammatory genes.

INTERACTION WITH TRANSCRIPTION FACTORS

GR may interact directly with other transcription factors, which bind to each other via so-called leucine zipper interactions [15,16]. This could be an important determinant of glucocorticoid responsiveness and is a key mechanism whereby glucocorticoids exert their anti-inflammatory actions [17]. This interaction was first demonstrated for the collagenase gene which is induced by the transcription factor activator protein-1 (AP-1), a heterodimer of Fos and Jun oncoproteins. AP-1 binds to specific DNA binding sites (TRE or TPA response element, TGACTCA). Glucocorticoids are potent inhibitors of collagenase gene transcription induced by tumour necrosis factor-α (TNF-α) and phorbol esters, which both activate AP-1. AP-1 forms a protein–protein complex with activated GR, and this prevents GR interacting with DNA and thereby reduces glucocorticoid responsiveness. In human lung TNF-α and phorbol esters increase AP-1 binding to DNA and this is inhibited by glucocorticoids [18,19].

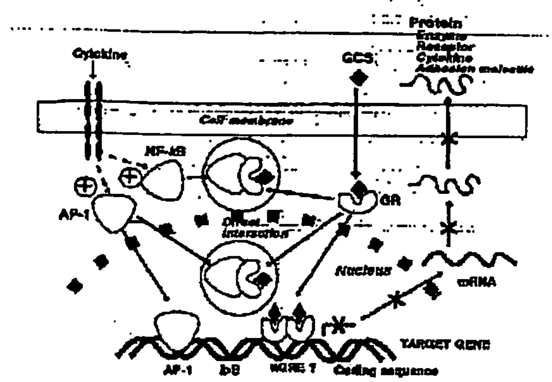

Figure 3 Direct interaction between the transcription factors AP-1 and NF-κB and the GR may result in mutual repression

In this way glucocorticoids may counteract the chronic inflammatory effects of cytokines which activate these transcription factors.

AP-1 may be important in regulating the expression of inflammatory genes in concert with other transcription factors, such as nuclear factor-κB (NF-κB).

NF-κB plays a critical part in regulating the expression of many inflammatory and immune genes and may play an amplifying role in the inflammatory process [20]. GR may interact with NF-κB in a similar manner by a direct protein–protein interaction, thus inhibiting the expression of a wide range of inflammatory genes [18,19,21–23] (Figure 3).

β_2-Adrenergic agonists, via cyclic AMP formation and activation of protein kinase A, result in the activation of the transcription factor CREB which binds to a cyclic AMP responsive element (CRE) on genes. A direct interaction between CREB and GR has been demonstrated [24]. β-Agonists increase CRE binding in human lung and epithelial cells *in vitro* and at the same time reduce GRE binding, suggesting that there may be a protein–protein interaction between CREB and GR within the nucleus [25]. However, in some cell lines cyclic AMP increases the transcriptional effects of glucocorticoids [26].

The interaction of GR with another family of transcription factors, signal transducers and activators of transcription (STATs), which are important for the signalling of many cytokines, has also been demonstrated. A positive interaction between GR and STAT5 and STAT6 has been shown, suggesting that glucocorticoids may enhance the effects of certain cytokines [27].

These interactions between activated GR and transcription factors occur within the nucleus, but protein–protein interactions may also occur in the cytoplasm [28].

EFFECTS ON CHROMATIN STRUCTURE

There has recently been increasing evidence that glucocorticoids may have effects on the chromatin structure. DNA in chromosomes is wound around

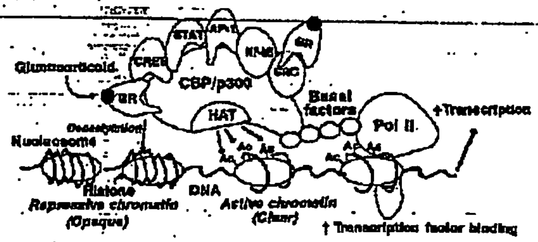

Figure 4 Effect of glucocorticoids on chromatin structure

Transcription factors such as STATs, AP-1 and NF-κB bind to co-activator molecules, such as CREB binding protein (CBP) or p300, which have intrinsic histone acetyltransferase (HAT) activity, resulting in acetylation (Ac) of histone residues. This leads to unwinding of DNA and allows increased binding of transcription factors resulting in increased gene transcription. Glucocorticoid receptors (GR) after activation by glucocorticoids may bind to a glucocorticoid receptor co-activator (SRC) which is bound to CBP and results in increased transcription. Activated GR, probably through binding to a co-repressor molecule, may also deacetylate histone, with increased coiling of DNA around histone, thus preventing transcription factor binding leading to gene repression.

histone molecules in the form of nucleosomes. Several transcription factors interact with large co-activator molecules, such as CREB binding protein (CBP) and the related p300, which bind to the basal transcription factor apparatus [29]. Several transcription factors have now been shown to bind directly to CBP, including AP-1, NF-κB and STATs [30–33]. Since binding sites on this molecule may be limited, this may result in competition between transcription factors for the limited binding sites available, so that there is an indirect rather than a direct protein–protein interaction (Figure 4). CBP also interacts with nuclear hormone receptors such as GR. These nuclear hormone receptors may interact with CBP and the basal transcriptional apparatus through binding to other nuclear co-activator proteins, including glucocorticoid receptor co-activator-1 (SRC-1) [34,35], transcription factor intermediary factor-2 (TIF-2) or glucocorticoid receptor interacting protein-1 (GRIP-1) [36]. A newly described nuclear protein called p300/CBP co-integrator-associated protein (p/CIP) appears to be particularly important in the binding of several nuclear receptors to CBP/p300 [37]. These nuclear activator proteins associate with nuclear receptors via a common sequence, LXXLL (where L is lysine and X is any amino acid) [38].

DNA is wound around histone proteins to form nucleosomes and the chromatin fibre in chromosomes. At a microscopic level that chromatin may become dense or opaque due to the winding or unwinding of DNA around the histone core. CBP and p300 have histone acetylation activity which is activated by the binding of transcription factors such as AP-1 and NF-κB [39]. Acetylation of histone residues results in unwinding of DNA coiled around the histone core, thus opening up the chromatin structure, which allows transcription factors to bind more readily, thereby increasing transcription (Figure 4). Repression of genes reverses this process by histone deacetylation [40]. The process of deacetylation involves the binding of hormone or vitamin receptors to co-repressor molecules such as nuclear receptor co-repressor (N-CoR), which forms a complex with another repressor molecule, Sin3, and a histone deacetylase [41,42]. Deacetylation of histone increases the winding of DNA round histone residues, resulting in dense chromatin structure and reduced access of transcription factors to their binding sites, thereby leading to repressed transcription of inflammatory genes. Activated GR may bind to several transcription co-repressor molecules that associate with proteins with histone deacetylase activity, with consequent repression of inflammatory genes by the mechanism described [40] (Figure 4).

TARGET GENES IN INFLAMMATION CONTROL

Glucocorticoids may control inflammation by inhibiting many aspects of the inflammatory process through increasing the transcription of anti-inflammatory genes and decreasing the transcription of inflammatory genes [2,17] (Table 1).

Anti-inflammatory proteins

Glucocorticoids may suppress inflammation by increasing the synthesis of several anti-inflammatory proteins. Glucocorticoids increase the synthesis of lipocortin-1, a 37 kDa protein that has an inhibitory effect on phospholipase A_2 (PLA_2), and therefore may inhibit the production of lipid mediators. Glucocorticoids induce the formation of lipocortin-1 in several cells and recombinant lipocortin-1 has acute anti-inflammatory properties [43]. However, glucocorticoids do not induce lipocortin-1 expression in many cell types and, indeed, the inhibitory effect of lipocortin on PLA_2 is largely an artefact and due to depletion of membrane phospholipids [44].

Glucocorticoids also increase the synthesis of se-

Table 1 Effect of glucocorticoids on gene transcription

Increased transcription
Lipocortin-1
β_2-Adrenoceptor
Secretory leucocyte inhibitory protein
Clara cell protein (CC10)
IL-1 receptor antagonist
IL-1R2 (decoy receptor)
IκB-α
Decreased transcription
Cytokines
(IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-11, IL-12, IL-13, TNF-α, GM-CSF, stem cell factor)
Chemokines
(IL-8, RANTES, MIP-1α, MCP-1, MCP-3, MCP-4, eotaxin)
iNOS
COX-2
Cytoplasmic PLA_2
Endothelin-1
NK_1-receptors, NK_2-receptors
Adhesion molecules (ICAM-1, E-selectin)

cretory leucocyte protease inhibitor in human airway epithelial cells by increasing gene transcription [45]. Secretory leucocyte protease inhibitor is the predominant antiprotease in airways and may be important in reducing airway inflammation by counteracting inflammatory enzymes such as tryptase. Clara cell protein (CC10), a 10 kDa protein secreted by epithelial cells which has anti-inflammatory and immunomodulatory properties, is also increased by glucocorticoids [46].

Interleukin (IL)-1 receptor antagonist is a cytokine that blocks the binding of IL-1 to its receptors. Its synthesis is increased by glucocorticoids, thus counteracting the effect of the pro-inflammatory cytokine IL-1. Therefore, treatment of asthmatic patients with inhaled glucocorticoids results in an increased expression of IL-1 receptor antagonist in airway epithelial cells *in vitro* and *in vivo* [47,48]. IL-1 interacts with two types of surface receptor, designated IL-1R1 and IL-1R2. The inflammatory effects of IL-1β are mediated exclusively via IL-1R1, whereas IL-1R2 has no signalling activity, but binds IL-1 and therefore acts as a 'molecular decoy' that interferes with the actions of IL-1. Glucocorticoids are potent inducers of this decoy IL-1 receptor and result in release of a soluble form of the receptor, thus reducing the functional activity of IL-1 [49].

IL-10 is an anti-inflammatory cytokine secreted predominantly by macrophages which inhibits the transcription of many pro-inflammatory cytokines, chemokines and inflammatory enzymes, and this appears to be mediated, at least in part, via an inhibitory effect on NF-κB [50]. IL-10 secretion by alveolar macrophages may be impaired in patients with asthma, resulting in increased macrophage cytokine secretion [51]. Glucocorticoid treatment in patients with asthma increases IL-10 secretion by these cells, although this appears to be an indirect effect, since treatment of alveolar macrophages *in vitro* with glucocorticoids tends to decrease IL-10 secretion [51].

NF-κB is regulated by the inhibitory protein IκB to which it is bound in the cytoplasm [20]. There is some evidence that glucocorticoids increase the synthesis and transcription of the predominant form of IκB, IκB-α, in mononuclear cells and T-lymphocytes, thus terminating the activation of NF-κB [52,53], but this has not been seen in other cell types [54–56]. The IκB-α gene does not appear to have any GRE consensus sequence, so any effect of glucocorticoids is probably mediated via other transcription factors.

In epithelial cells glucocorticoids also increase the expression of the enzyme neutral endopeptidase, which degrades inflammatory peptides such as substance P, bradykinin and endothelin-1 [57]. Patients with asthma treated with inhaled glucocorticoids have a higher level of neutral endopeptidase expression than untreated patients [58].

β_2-Adrenoceptors

Glucocorticoids increase the expression of β_2-adrenoceptors by increasing the rate of transcription and the human β_2-receptor gene has three potential GREs [59]. Glucocorticoids double the rate of β_2-receptor gene transcription in human lung *in vitro*, resulting in increased expression of β_2-receptors [60]. Using autoradiographic mapping and *in situ* hybridization in animals to localize the increase in β_2-receptor expression, there appears to be an increase in all cell types, including airway epithelial cells and airway smooth muscle, after chronic glucocorticoid treatment [61]. This may be relevant in asthma as it may prevent down-regulation in response to prolonged treatment with β_2-agonists. In rats glucocorticoids prevent the down-regulation and reduced transcription of β_2-receptors in response to chronic β-agonist exposure [61], although inhaled glucocorticoids have not been shown to prevent tolerance to the bronchoprotective effects of an inhaled β_2-agonist [62].

Cytokines

Although it is not yet possible to be certain of the most critical aspects of glucocorticoid action in chronic inflammatory diseases such as asthma, it is likely that their inhibitory effects on cytokine synthesis are of particular importance. Glucocorticoids inhibit the transcription of several cytokines that are relevant in inflammatory diseases, including IL-1β, IL-2, IL-3, IL-6, IL-11, TNF-α, GM-CSF and chemokines that attract inflammatory cells to the site of inflammation, including IL-8, RANTES, MCP-1, MCP-3, MCP-4, MIP-1α and eotaxin. In allergic inflammation the expression of cytokines IL-4 (critical for IgE synthesis) and IL-5 (critical for eosinophilic inflammation) is also inhibited by glucocorticoids. These inhibitory effects were at one time thought to be mediated directly via interaction of GR with a negative GRE in the upstream promoter sequence of the cytokine gene, resulting in gene repression. However, there is no negative GRE consensus sequence in the upstream promoter region of any of these cytokine genes, suggesting that glucocorticoids inhibit transcription indirectly. For example, the 5′-promoter sequence of the human IL-2 gene has no GRE consensus sequences, yet glucocorticoids are potent inhibitors of IL-2 gene transcription in T-lymphocytes. Transcription of the IL-2 gene is predominantly regulated by a cell-specific transcription factor, nuclear factor of activated T-cells (NF-AT), which is activated in the cytoplasm on T-cell receptor stimulation via calcineurin. A nuclear factor is also necessary for increased activation and this factor has been identified as AP-1, which binds directly to NF-AT to form a transcriptional complex [63]. Glucocorticoids therefore inhibit IL-2 gene transcription indirectly by binding to AP-1, thus preventing increased transcription due to NF-AT [64]. Inhibition of IL-5 gene transcription may involve a similar mechanism [65]. Another example of a cytokine gene negatively regulated by glucocorticoids that does not have a GRE in its promoter region is RANTES, which is regulated predominantly by NF-κB and AP-1 [66]. Glucocorticoids therefore appear to inhibit

cytokine gene expression by inhibiting transcription factors that regulate their expression, rather than by binding to their promoter regions.

There may be marked differences in the response of different cells and of different cytokines to the inhibitory action of glucocorticoids and this may be dependent on the relative abundance of transcription factors. Thus in alveolar macrophages and peripheral blood monocytes GM-CSF secretion is more potently inhibited by glucocorticoids than IL-1β or IL-6 secretion [67]. This might be explained by the need for different combinations of transcription factor activation for optimal gene transcription, so that glucocorticoid sensitivity may be determined by the particular combination of transcription factors needed and their propensity for activation in different cell types.

Inflammatory enzymes

Nitric oxide (NO) synthase is inducible by pro-inflammatory cytokines, resulting in increased NO production. NO may increase blood flow and plasma exudation and may amplify the inflammatory response. In the airways NO may contribute to the plasma exudation seen in asthma and other inflammatory diseases, and may amplify eosinophilic inflammation in asthma by tipping the immune balance in favour of Th2 lymphocytes that secrete IL-4 and IL-5, by acting as a chemotactic agent for eosinophils and by increasing eosinophil survival [68,69]. The induction of the inducible form of NO synthase (iNOS) is potently inhibited by glucocorticoids [70]. In cultured human pulmonary epithelial cells pro-inflammatory cytokines result in increased expression of iNOS and increased NO formation due to increased transcription of the iNOS gene and this is inhibited by glucocorticoids [71]. There is no negative GRE in the promoter sequence of the iNOS gene, but NF-κB appears to be the most important transcription factor in regulating iNOS gene transcription [72]. Since TNF-α, IL-1β and oxidants activate NF-κB in airway epithelial cells, this accounts for their activation of iNOS expression. Glucocorticoids may therefore prevent induction of iNOS by inhibiting NF-κB, thereby inhibiting transcription. The increased expression of iNOS in the airways of patients with asthma results in an increase in the level of NO in the exhaled air [73] and this is inhibited by inhaled glucocorticoids [74].

Glucocorticoids inhibit the synthesis of several inflammatory mediators implicated in inflammation through an inhibitory effect on enzyme induction. Glucocorticoids inhibit the induction of the gene coding for COX-2 in monocytes and epithelial cells and this also appears to be via NF-κB activation [75–77] and by a post-transcriptional effect on mRNA stability [14]. Glucocorticoids also inhibit the gene transcription of a form of phospholipase A_2 ($cPLA_2$) induced by cytokines [77]. However, glucocorticoids do not appear to modulate expression of the 5′-lipoxygenase and studies of cysteinyl-leukotriene formation in patients with asthma *in vivo* indicate that doses of oral or inhaled glucocorticoids that are effective clinically do not significantly reduce the excretion of leukotriene E_4, the major stable metabolite of leukotriene D_4 [78].

Glucocorticoids also inhibit the synthesis of endothelin-1 in lung [79] and airway epithelial cells and this effect may also be via inhibition of transcription factors that regulate its expression [80].

Inflammatory receptors

Glucocorticoids also decrease the transcription of genes coding for certain receptors that are involved in the inflammatory process. Thus the NK_1-receptor, which mediates the inflammatory effects of tachykinins in the airways, has increased gene expression in asthma [81]. This may be inhibited by glucocorticoids through an interaction with AP-1, as the NK_1 receptor gene promoter region has no GRE, but has an AP-1 response element [82]. Similarly NK_2-receptor expression is also reduced by glucocorticoids [83].

Cell survival

Glucocorticoids markedly reduce the survival of certain inflammatory cells such as eosinophils and T-lymphocytes. Eosinophil survival is dependent on the presence of cytokines such as IL-5 and GM-CSF. Exposure to glucocorticoids blocks the effects of these cytokines and leads to programmed cell death or apoptosis [84]. Glucocorticoids also promote apoptosis of T-lymphocytes. The molecular mechanism of action of glucocorticoids in increasing apoptosis in eosinophils and T-lymphocytes is uncertain and there are many potential sites of action, including effects on endogenous inhibitors of the apoptotic pathway. In contrast, glucocorticoids decrease apoptosis and therefore increase the survival of neutrophils [85,86]. The molecular mechanisms that account for the opposing effects of glucocorticoids on these two types of granulocyte are not yet certain.

Adhesion molecules

Adhesion molecules play a key role in the trafficking of inflammatory cells to sites of inflammation. The expression of many adhesion molecules on endothelial cells is induced by cytokines and glucocorticoids may lead indirectly to a reduced expression via their inhibitory effects on cytokines such as IL-1β and TNF-α. Glucocorticoids may also have a direct inhibitory effect on the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and E-selectin at the level of gene transcription [87]. ICAM-1 expression in bronchial epithelial cell lines and monocytes is inhibited by glucocorticoids [88].

EFFECTS ON CELL FUNCTION

Glucocorticoids may have direct inhibitory actions on many inflammatory and structural cells involved in inflammation.

Macrophages

Glucocorticoids inhibit the release of inflammatory mediators and cytokines from alveolar macrophages *in vitro* [67,89], although their effect after inhalation *in vivo* is modest [90]. Glucocorticoids may be more effective in inhibiting cytokine release from alveolar macrophages than in inhibition of lipid mediators and reactive oxygen species *in vitro* [91,92]. Inhaled glucocorticoids reduce the secretion of chemokines and pro-inflammatory cytokines from alveolar macrophages in patients with asthma, whereas the secretion of IL-10 is increased [51]. Oral prednisone inhibits the increased gene expression of IL-1β in alveolar macrophages obtained by bronchoalveolar lavage from patients with asthma [93].

Eosinophils

Glucocorticoids have a direct inhibitory effect on mediator release from eosinophils, although they are only weakly effective in inhibiting secretion of reactive oxygen species and eosinophil basic proteins [94,95]. Glucocorticoids inhibit the permissive action of GM-CSF and IL-5 on eosinophil survival [96,97]. The increased apoptosis contributes to the reduction in airway eosinophils seen with glucocorticoid therapy. One of the best described actions of glucocorticoids in asthma is a reduction in circulating eosinophils, which may reflect an action on eosinophil production in the bone marrow. In patients with asthma there is an increase in the proportion of low-density eosinophils in the circulation, which may reflect an effect of cytokines [98]. Inhaled glucocorticoids inhibit the increase in circulating eosinophil count at night in patients with nocturnal asthma and also reduce plasma concentrations of eosinophil cationic protein [99]. After inhaled glucocorticoids there is a marked reduction in the number of low-density eosinophils, presumably reflecting inhibition of cytokine production in the airways [100].

T-lymphocytes

T-lymphocytes play a key role in orchestrating chronic inflammation and glucocorticoids are very effective in inhibiting activation, proliferation and survival of these cells, and in blocking the release of lymphokines such as IL-2, IL-3, IL-4, IL-5, IL-13 and GM-CSF, which are likely to play an important role in the recruitment and survival of inflammatory cells.

Mast cells

While glucocorticoids do not appear to have a direct inhibitory effect on mediator release from mast cells [101], chronic inhaled glucocorticoid treatment is associated with a marked reduction in mucosal mast cell number in airways [102]. This may be linked to a reduction in IL-3 and stem cell factor production, which is necessary for mast cell expression at mucosal surfaces. Mast cells also secrete various cytokines (TNF-α, IL-4, IL-5, IL-6, IL-8), but whether this is inhibited by glucocorticoids is not yet certain.

Dendritic cells

Dendritic cells in the epithelium of the respiratory tract appear to play a critical role in antigen presentation in the lung as they have the capacity to take up allergen, process it into peptides and present it via MHC molecules on the cell surface to uncommitted T-lymphocytes [103]. In experimental animals the number of dendritic cells is markedly reduced by systemic and inhaled glucocorticoids, thus dampening the immune response in the airways [104]. Topical glucocorticoids markedly reduce the number of dendritic cells in the nasal mucosa [105], and it is likely that a similar effect would be seen in airways.

Neutrophils

Neutrophils, which are not prominent in the biopsies of patients with asthma, are not very sensitive to the effects of glucocorticoids. Indeed systemic glucocorticoids increase peripheral neutrophil counts, which may reflect the increased survival time due to an inhibitory action of neutrophil apoptosis [85,86].

Endothelial cells

GR gene expression in the airways is most prominent in endothelial cells of the bronchial circulation and airway epithelial cells [106]. Glucocorticoids do not appear to directly inhibit the expression of adhesion molecules, although they may inhibit cell adhesion indirectly by suppression of cytokines involved in the regulation of adhesion molecule expression. Glucocorticoids may have an inhibitory action on airway microvascular leak induced by inflammatory mediators [107,108]. This appears to be a direct effect on postcapillary venular epithelial cells. The mechanism for this antipermeability effect has not been fully elucidated, but there is evidence that synthesis of a 100 kDa protein distinct from lipocortin-1 termed vasocortin may be involved [109]. Although there have been no direct measurements of the effects of glucocorticoids on airway microvascular leakage in asthmatic airways, regular treatment with inhaled glucocorticoids decreases the elevated plasma proteins found in bronchoalveolar lavage fluid of patients with stable asthma [110].

Epithelial cells

Epithelial cells may be an important source of inflammatory mediators in asthmatic airways and may drive and amplify the inflammatory response in the airways [111,112]. Airway epithelium may be one of the most important targets for inhaled glucocorticoids in asthma [3,113]. Glucocorticoids inhibit the increased transcription of the IL-8 gene induced by TNF-α in cultured human airway epithelial cells *in*

vitro [114,115] and the transcription of the RANTES gene in epithelial cells [116,117]. Inhaled glucocorticoids inhibit the increased expression of GM-CSF and RANTES in the epithelium of patients with asthma [111,118,119].

Glucocorticoids decrease the transcription of other inflammatory proteins in airway epithelial cells, including iNOS, COX-2, $cPLA_2$ and endothelin-1 [71,75,80]. Airway epithelial cells may be the key cellular target of inhaled glucocorticoids; by inhibiting the transcription of several inflammatory genes inhaled glucocorticoids may reduce inflammation in the airway wall.

Mucus secretion

Glucocorticoids inhibit mucus secretion in airways and this may be by a direct action on submucosal gland cells [120]. Recent studies suggest that glucocorticoids may also inhibit the expression of mucin genes such as *MUC2* and *MUC5A* [121]. In addition there are indirect inhibitory effects due to the reduction in inflammatory mediators that stimulate increased mucus secretion.

Neurogenic inflammation

Neurogenic inflammation, due to release of neuropeptides such as tachykinins from sensory nerves, may amplify inflammatory responses. Glucocorticoids may inhibit several aspects of neurogenic inflammation, including the synthesis of tachykinins by repression of the preprotachykinin-A gene [122], reduced expression of tachykinin receptors [81,83] and by increasing expression of neutral endopeptidase which degrades tachykinins [57].

GLUCOCORTICOID RESISTANCE

Although glucocorticoids are highly effective in the control of chronic inflammatory or immune diseases, a small proportion of patients will fail to respond even to high doses of oral glucocorticoids. This has been most extensively studied in asthma, as it is easier to assess the clinical response to glucocorticoids in this condition [123–125], although resistance to the therapeutic effects of glucocorticoids is also recognized in other inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease [126]. Glucocorticoid-resistant patients, although uncommon, present considerable management problems. Recognition of patients with glucocorticoid resistance is important, as elucidation of the molecular mechanisms may contribute to our understanding of glucocorticoid action and inflammatory disease mechanisms.

Clinical features of glucocorticoid-resistant asthma

Glucocorticoid resistance in asthma was first described by Schwartz et al. [127] in 1968 in six patients with asthma who did not respond clinically to high doses of systemic glucocorticoids and in whom there was also a reduced eosinopenic response. Larger groups of patients with chronic asthma who were glucocorticoid resistant were subsequently identified [128]. These patients failed to improve their mean peak expiratory flow by > 15% after taking prednisolone, 20 mg daily for at least 7 days. These patients are not Addisonian and do not suffer from the abnormalities in sex hormones described in familial glucocorticoid resistance (see below). Plasma cortisol and adrenal suppression in response to exogenous cortisol is normal [129]. Complete glucocorticoid resistance in asthma is very rare, but reduced responsiveness is more common, so that oral glucocorticoids are needed to control asthma adequately (steroid-dependent asthma).

Glucocorticoid-resistant (GCR) asthma is defined by a failure to improve lung function by > 15% after treatment with 30 to 40 mg of prednisolone for 2 weeks. These patients show the typical diurnal variability in peak expiratory flow and bronchodilate with inhaled β_2-agonists. Fibre-optic biopsies in patients with GCR asthma show the typical eosinophilic inflammation observed in patients with glucocorticoid-sensitive (GCS) asthma [130].

Mechanisms of glucocorticoid resistance

There may be several mechanisms for resistance to the effects of glucocorticoids. Although a family history of asthma is more common in patients with GCR than GCS asthma, little is known about its inheritance. It is possible that a certain proportion of the population has glucocorticoid resistance which only becomes manifest when they develop a severe immunological or immune disease that requires glucocorticoid therapy. Resistance to the inflammatory and immune effects of glucocorticoids should be distinguished from the rare familial glucocorticoid resistance, where there is an abnormality of glucocorticoid binding to GR.

Familial glucocorticoid resistance. Familial glucocorticoid resistance (FGR) is an extremely rare syndrome characterized by high circulating levels of cortisol without signs or symptoms of Cushing's syndrome [131]. Clinical manifestations, which may be absent, are due to an excess of non-glucocorticoid adrenal steroids, stimulated by high adrenocorticotropic hormone levels, resulting in hypertension with hypokalaemia and/or signs of androgen excess (usually hirsutism and menstrual abnormalities in females). Inheritance may be recessive, but only about 12 cases have so far been reported. Several abnormalities in GR function have been described in peripheral blood leucocytes or fibroblasts from these patients. These include a decreased affinity of GR for cortisol, a reduced number of GRs, GR thermolability and an abnormality in the binding of the GR complex to DNA. The molecular basis of the disease in patients with a reduction in GR appears to be a point mutation

in the glucocorticoid binding domain of GR [132]. Glucocorticoid resistance may occur in certain malignancies and this may be due to abnormal expression of GR. Thus, in multiple myeloma, resistance to the inhibitory effect of glucocorticoids is associated with the expression of a truncated form of GR mRNA with reduced stability, resulting in a shortened form of GR with markedly reduced glucocorticoid binding [133].

Resistance to anti-inflammatory actions of glucocorticoids. Resistance to the anti-inflammatory and immunomodulatory effects of glucocorticoids differs from the FGR described above, as it is not associated with high circulating concentrations of cortisol or adrenocorticotropic hormone, and is not accompanied by hypertension, hypokalaemia or androgen excess [124]. Furthermore, these patients are not Addisonian and show normal adrenal suppression [129]. This suggests that any abnormality is unlikely to be due to those described for FGR in the glucocorticoid binding domain of GR. Indeed, chemical mutational analysis of GR has failed to demonstrate any major abnormality in predicted structure in GCR compared with GCS asthma [134]. Glucocorticoid resistance may be *primary* (inherited or acquired of unknown cause) or *secondary* to some factor that may reduce glucocorticoid responsiveness (glucocorticoids themselves, cytokines, β-adrenergic agonists). There are several possible mechanisms for a reduced anti-inflammatory response to glucocorticoids.

Pharmacokinetic abnormalities. The initial suggestion of Schwartz et al. [127] was that defective responses to glucocorticoids were due to increased clearance of the glucocorticoid, resulting in reduced clinical and eosinopenic response. There is no evidence for altered bioavailability or plasma clearance of prednisolone or methylprednisolone in patients with GCR asthma [135,136]. Metabolism of glucocorticoids may be increased by induction of cytochrome *P*-450 enzymes in response to certain drugs, which may thus lead to a secondary glucocorticoid resistance [137].

Antibodies to lipocortin-1. Some anti-inflammatory effects of glucocorticoids may be due to induction of lipocortin-1 [43]. In some patients with GCR rheumatoid arthritis, autoantibodies to lipocortin-1 have been described [138]. However, two independent studies have failed to demonstrate the presence of IgG or IgM lipocortin-1 antibodies in either GCR or steroid-dependent asthma [139,140].

Cellular abnormalities. Glucocorticoid resistance has been documented *in vitro*, with reduced suppression of activation antigens and cytokines in monocytes and T-lymphocytes from patients with GCR asthma [136,141–145]. There is no difference in the proportion of $CD4^+$ and $CD8^+$ T-lymphocytes in GCR patients, although there is increased expression of CD25 (IL-2 receptor) in GCR compared with GCS patients, indicating a greater degree of immune activation [145]. These studies in circulating leucocytes suggest that the defect in glucocorticoid responsiveness extends outside the respiratory tract and is therefore unlikely to be explained entirely by inflammatory changes in the airways. In patients with GCR asthma a reduced blanching response to topical glucocorticoids applied to the skin further indicates that there is a generalized abnormality that is unlikely to be secondary to local cytokine production [146]. In patients with GCS asthma there was suppression of the cutaneous tuberculin response after treatment for 2 weeks with oral prednisolone associated with reduced numbers of macrophages, eosinophils and activated T-lymphocytes, but this was not observed in GCR patients [147].

Abnormality in GR function. In FGR there is a structural abnormality in GR that results in reduced glucocorticoid binding affinity. In GCR asthma either no difference in GR affinity and receptor density or a relatively small reduction in GR affinity has been reported [135,145,148,149]. Two types of glucocorticoid resistance have been described: a reduced affinity of GR binding confined to T-lymphocytes which reverts to normal after 48 h in culture, and a much less common reduction in GR density (in only 2/17 GCR patients), which does not normalize with prolonged incubation [149]. This suggests that there may be different types of GCR asthma. The small reduction in GR affinity is unlikely to be of functional significance and is not associated with elevated plasma cortisol, unlike patients with FGR. The small reduction in GR affinity may be secondary to cytokine exposure, since the normalization of GR affinity *in vitro* is prevented by a combination of IL-2 and IL-4 [149] and this combination of cytokines reduces the binding affinity in nuclear GR in T-lymphocytes, although either cytokine alone has no effect [150]. The IL-4-like cytokine IL-13 has a similar effect and is effective alone [151]. This suggests that glucocorticoid resistance may occur in the airways of patients with asthma as a secondary phenomenon due to the local production of cytokines. In patients with GCR asthma there is a significant increase in the numbers of bronchoalveolar lavage cells expressing IL-2 and IL-4 mRNA compared with patients with GCS asthma, but no difference in interferon-γ mRNA-positive cells. After oral prednisone for 1 week there is a reduction in IL-4-expressing cells and a rise in interferon-γ-positive cells in GCS asthma, whereas in GCR asthma there is no fall in IL-4-positive cells and a fall in interferon-γ-positive cells [130]. This may indicate that there are different patterns of cytokine release which may contribute to glucocorticoid resistance. Although this may account for the increased requirement for glucocorticoids in more severe asthma, it is unlikely to explain the reduced glucocorticoid response seen in circulating mononuclear cells and in the skin of patients with no response to oral glucocorticoids.

The recognition that there is a splice variant of GR, GR-β, that does not bind glucocorticoids, but binds to GRE [4], has suggested that this might be a mechanism of glucocorticoid resistance if GR-β is produced in excess. There is some evidence for an increase in GR-β is induced by inflammatory cytokines and there is an increase in GR-β-producing cells in GCR patients [152]. However, it appears to be unlikely that GR-β

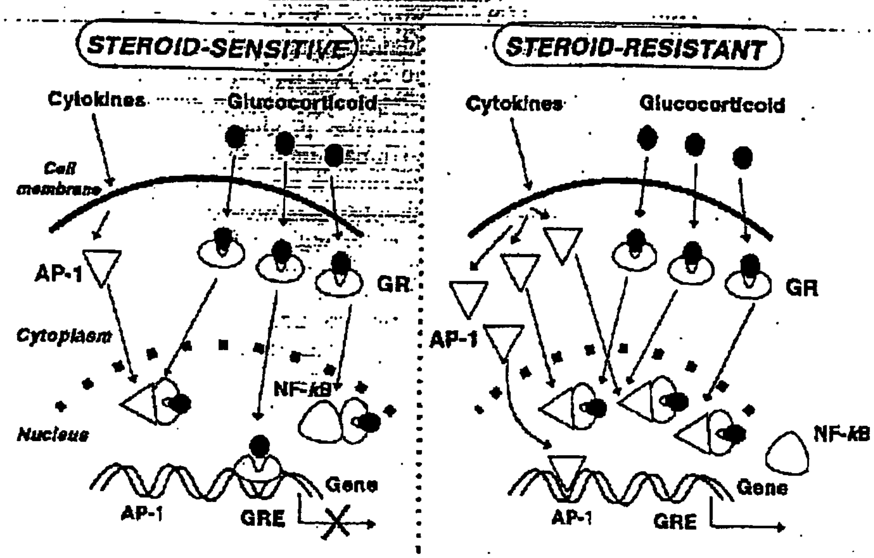

Figure 5 Proposed mechanism of primary glucocorticoid resistance in asthma

Increased activation of AP-1 results in the complexing of GRs, thus preventing the anti-inflammatory action of glucocorticoids, either through binding to GREs or through inhibition of NF-κB.

has any functional effect on GRE binding of GR-α [153].

There is a marked reduction in GR–GRE binding in the peripheral blood mononuclear cells of patients with GCR asthma and Scatchard analysis has demonstrated a marked reduction in GR available for DNA binding compared with cells from patients with GCS asthma [154].

Interaction between GR and transcription factors. In the peripheral blood mononuclear cells of patients with GCS asthma and normal control subjects the phorbol ester PMA, which activates AP-1, results in reduced GRE binding. This inhibitory effect is significantly reduced in the peripheral blood mononuclear cells of patients with GCR asthma, indicating an abnormality in the interaction between GR and AP-1 [155]. This defect does not appear to apply to the other transcription factors, NF-κB and CREB, that also interact with GR [155]. The abnormality in the interaction between GR and AP-1 is unlikely to be due to a defect in GR, since the protein sequence of GR in patients with GCR asthma appears to be normal [134]. It is more likely to be due to a defect in AP-1 or its activation. Indeed, activation of c-Fos by phorbol esters is potentiated in the cells of patients with GCR compared with GCS asthma [156], and preliminary evidence suggests that one of the key enzymes involved in activation of AP-1, namely Jun N-terminal kinase (JNK) is abnormally activated in these patients [157]. The increased basal and cytokine-induced AP-1 activity may lead to consumption of GR, so that glucocorticoids are not able to suppress the inflammatory response, either through interacting with GRE or with other transcription factors such as NF-κB (Figure 5).

An abnormality in AP-1 may also account for the selective resistance to the effects of glucocorticoid in GCR asthma, since AP-1 is more likely to be important in the regulation of some genes than in others. It would also explain why resistance is seen to the anti-inflammatory effects but not to the endocrine or metabolic effects of glucocorticoids, since such resistance can only arise when AP-1 is activated at the inflammatory site, whereas the hormonal effects of glucocorticoids at uninflamed sites will not be impaired. Furthermore, there may also be differences in the glucocorticoid resistance of different target cells, depending upon the relative balance of transcription factors.

Secondary glucocorticoid resistance

Although complete glucocorticoid resistance is uncommon, there may be a spectrum of glucocorticoid responsiveness in inflammatory diseases. This may reflect several mechanisms that are secondary either to disease activity itself or to the effects of therapy.

Down-regulation of GR. Down-regulation of GR in circulating lymphocytes after oral prednisolone has been demonstrated in normal individuals [158]. Whether high local concentrations of inhaled glucocorticoids reduce GR expression in surface cells of the airway, such as epithelial cells, is not yet certain, although patients with asthma treated with inhaled glucocorticoids do not appear to have a reduced expression of GR in the airways [106]. It is possible that certain individuals may be more susceptible to the effects of down-regulation. If effective GR density is reduced by direct interaction with other transcription factors, such as AP-1 and NF-κB, then the down-regulating effect of glucocorticoids on GR would be expected to have a greater functional consequence.

Effects of cytokines. Several pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α, activate AP-1 and NF-κB in human lung [18,159]. As all these cytokines are secreted in asthmatic inflammation, this suggests that these transcription factors will be activated in the cells of asthmatic airways. These activated transcription factors may then form protein–protein complexes with activated GR, both in the cytoplasm and within the nucleus, thus reducing the number of effective GRs and thereby decreasing glucocorticoid responsiveness [17]. In a model *in vitro* system increased expression of c-Fos or c-Jun oncoproteins prevents the activation of mouse mammary tumour virus promoter by GR, thus creating a model of glucocorticoid resistance [160]. Addition of recombinant c-Jun or c-Fos proteins to partially purified GR results in inhibition of DNA binding [160]. Phorbol esters, which activate AP-1, result in attenuation of glucocorticoid-mediated gene activation [161]. Any reduction in glucocorticoid responsiveness would be greater as the intensity of asthmatic inflammation increased and may contribute, for example, to the failure of oral or intravenous glucocorticoids to control acute exacerbations of asthma. Once the inflammation is brought under control with large doses of oral glucocorticoids, glucocorticoid responsiveness increases again so that lower doses of inhaled or oral glucocorticoids are needed to control the inflammation.

Increased resistance may also be due to the effects of cytokines on GR function, since high concentrations of IL-2 and IL-4 have been shown to reduce GR affinity in T-lymphocytes *in vitro* [150]. This effect would only be seen in mucosal T-lymphocytes of patients with severe asthma and it is therefore difficult to obtain evidence to support this possibility.

Effect of β_2-agonists. High concentrations of β_2-agonists activate CREB in rat and human lung and in inflammatory cells via an increase in cyclic AMP concentration [19,25]. This results in reduced GRE binding due to the formation of GR–CREB complexes [25]. This predicts that high concentrations of β_2-agonists would induce glucocorticoid resistance. In patients with asthma, while 3 weeks of treatment with an inhaled glucocorticoid blocked the airway response to inhaled allergen, concomitant treatment with inhaled glucocorticoid and a relatively large dose of inhaled β-agonist appeared to provide no significant protection against allergen challenge [162]. This suggests that high doses of an inhaled β_2-agonist might interfere with the anti-asthma effect of inhaled glucocorticoids. It is possible that some patients who use very high doses of inhaled β_2-agonists (over two canisters per month of metered-dose inhalers or regular nebulized doses) may develop a degree of glucocorticoid resistance that is overcome by increasing the dose of inhaled or oral glucocorticoid. This may account for some of the deleterious effects of high-dose β-agonists on asthma mortality and morbidity [163]. The use of high doses of nebulized β_2-agonists in the treatment of acute exacerbations of asthma may result in resistance to the effects of high-dose intravenous glucocorticoids. Glucocorticoid responsiveness might be restored by reducing the dose of inhaled β_2-agonists.

THERAPEUTIC IMPLICATIONS

Greater understanding of the molecular mechanism whereby glucocorticoids suppress inflammation has opened up the potential for improvement in glucocorticoids and the development of novel anti-inflammatory drugs.

New glucocorticoids

The recognition that most of the anti-inflammatory effects of glucocorticoids are mediated by repression of transcription factors (transrepression), whereas the endocrine and metabolic effects of steroids are likely to be mediated via GRE binding (transactivation) has led to a search for novel corticosteroids that selectively transrepress, thus reducing the potential risk of systemic side effects. Since corticosteroids bind to the same GR, this seems at first to be an unlikely possibility, but while GRE binding involved a GR homodimer, interaction with transcription factors AP-1 and NF-κB involves only a single GR. A separation of transactivation and transrepression has been demonstrated using reporter gene constructs in transfected cells using selective mutations of GR [164]. Furthermore, some steroids, such as the antagonist RU486, have a greater transrepression than transactivation effect. Indeed, the topical steroids used in asthma therapy today, such as fluticasone propionate and budesonide, appear to have more potent transrepression than transactivation effects, which may account for their selection as potent anti-inflammatory agents [165]. Recently, a novel class of steroids has been described in which there is potent transrepression with relatively little transactivation. These 'dissociated' steroids, including RU24858 and RU40066, have anti-inflammatory effects *in vivo* [166]. This suggests that the development of steroids with a greater margin of safety is possible and may predict the development of oral steroids that are safe to use in inflammatory diseases.

NF-κB inhibitors

Since NF-κB appears to mediate many of the anti-inflammatory effects of glucocorticoids, this has led to a search for specific inhibitors of this transcription factor or its activating pathways [20,167]. Antioxidants have the ability to block activation of NF-κB in response to a wide variety of stimuli, and drugs such as pyrrolidine dithiocarbamate have proved useful for *in vitro* studies, but are too toxic for *in vivo* development [168]. Spin-trap antioxidants may be more effective since they work at an intracellular level

[169]. However, antioxidants do not block all of the effects of NF-κB and this may require the development of novel drugs.

Some naturally occurring NF-κB inhibitors have already been identified. Thus gliotoxin, derived from *Aspergillus*, is a potent NF-κB inhibitor which appears to be relatively specific [170]. The anti-inflammatory cytokine IL-10 also has an inhibitory effect on NF-κB, via an effect on IκB-α [50], and has been shown to be effective in management of chronic inflammatory diseases such as Crohn's disease, which is resistant to glucocorticoid therapy [171].

Novel approaches to inhibition of NF-κB would be to develop specific inhibitors of IκB kinases involved in the initial activation of NF-κB, to block the signal transduction pathways leading to activation of IκB kinases. Now that IκB kinases have been identified, it may be possible to screen and design specific inhibitors. It may also be possible to inhibit the activity of the enzymes responsible for its degradation of the IκB complex, although the proteasome has many other important functions and its inhibition is likely to produce severe side effects. Recently it has been possible to block NF-κB function by targeting of a specific enzyme (ubiquitin ligase) involved in conjugation of ubiquitin [172]. It may be more difficult to develop drugs to directly inhibit the components of NF-κB itself, but antisense oligonucleotides have been shown to be effective inhibitors *in vitro* and stable cell permeable phosphorothioate oligonucleotides are a therapeutic possibility in the future. Adenovirus-mediated gene transfer of IκB-α has been reported to inhibit endothelial cell activation [173].

It may be unwise, however, to block NF-κB for prolonged periods, as it plays such a critical role in immune and host defence responses. Targeted disruption ('knock-out') of p65 is lethal because of developmental abnormalities [174], whereas lack of p50 results in immune deficiencies and increased susceptibility to infection [175]. However, topical application of NF-κB inhibitors by inhalation may prove to be safe.

Drug Interactions

There are complex interactions between transcription factors, either directly or via co-activator molecules such as CBP. This might be exploited therapeutically by a combination of drugs which act on different transcript factors or pathways that may work together co-operatively. For example, NF-AT has a cytoplasmic component (NF-ATp) which is blocked by cyclosporin and tacrolimus (FK506), and a nuclear component (AP-1) which is blocked by glucocorticoids. Combining steroids and cyclosporin may therefore have a synergistic inhibitory effect on the expression of genes such as IL-2, IL-4 and IL-5. This has indeed been demonstrated for IL-2 in human T-lymphocytes, where a combination of both drugs has a much greater suppressive effect than either drug alone [176]. This suggests that a dose of cyclosporin A that is too low to give nephrotoxic side effects may be combined with an inhaled steroid, so that this synergistic interaction is confined to the airways.

Another interaction that may be exploited therapeutically is that between retinoic acid and steroids. Retinoic acid (vitamin A) binds to retinoic acid receptors which, like GR, bind to CBP. There appears to be a synergistic interaction between steroids and retinoic acid in repression of transcription factors such as NF-κB and AP-1, presumably because of competition for binding sites on CBP. A synergistic interaction between retinoic acid and steroids has been demonstrated in suppression of GM-CSF release from cultured epithelial cells, suggesting that retinoic acid may potentiate the anti-inflammatory effects of steroids [177]. Novel retinoic acid derivatives activate a subtype of retinoic acid receptor (RXR) which interacts with these transcription factors, and thus it may be possible to develop more selective retinoids for this purpose [178].

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